Field testing of indigenously synthesized sex pheromone for the management of *Phyllocnistis citrella* Stainton under central Indian conditions

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The objective of this study was to determine the optimum height and dose of the indigenously synthesized sex pheromone of citrus leaf miner, *Phyllocnistis citrella* Stainton for the management of citrus groves under central Indian conditions. The influence of pheromone dose (5 and 8 mg) and trap height (canopy and mid-canopy height) on the capture of *P. citrella* males was evaluated. The dose of 5 mg of synthetic sex pheromone placed at mid-canopy (1.55 m) height attracted the highest number of *P. citrella* males (667.15 ± 9.84 adults/trap/week) in <5 year-old *Citrus aurantifolia* (Christm) Swingle (acid lime) groves of ICAR-CCRI, Nagpur experimental farm. Further evaluation of the pheromone baited traps @ 10, 15 and 20 mg/lure in <5-year-old orchards of acid lime recorded significant maximum trap catch in orchards with 20 mg lure (4 traps/0.3 ha) with mean trap catch/month from 4, 3 and 2 traps/0.3 ha of 1659.76 ± 36.67, 950.19 ± 17.91 and 668.44 ± 8.78 adults in 2014–15 and 1400.17 ± 36.67, 873.64 ± 17.91, 446.91 ± 8.78 adults in 2015–16 respectively.

Keywords: Citrus groves, optimum height and dose, *Phyllocnistis citrella*, sex pheromone, semiochemicals.

CITRUS leaf miner (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) is an important pest of citrus in Asia, Australia, Africa, America and Europe[1]. A widespread Asian species[2,3], CLM was described from Calcutta, India[4]. *P. citrella* is the most serious pest among 27 major species of insects and mites attacking citrus, particularly during flushing seasons[5].

Damage is caused by the larvae mining inside the epidermal layer of young citrus leaves. In central India, citrus nurseries are found severely affected by leaf miner infestation, and more than 80% infestation has been reported from India[6]. Indirect impact of this insect pest is that it acts as a vector for the development of citrus canker by *Xanthomonas citri* Hasse by opening the leaf cuticle to infection, thereby augmenting infection[7,8]. Apart from the above, secondary effects of citrus leaf miner damage may also include leaf desiccation or invasion by fungi and bacteria[9]. Due to the feeding by CLM larvae, the leaf distorts and other insects such as the aphids (*Aphis gossypii* Glover), mealybugs (*Planococcus citri* (Risso), thrips (*Scirtothrips dorsalis* Hood) may continue feeding inside the mines on the damaged area[10].

During flushing season, new growth will continuously take place and hence, insecticide applications made at a particular time would not protect new growth that appears after the application. Young trees, especially in the initial period of establishment, will have gradual growth and might be retarded due to continuous feeding followed by defoliation by CLM. Due to its high migration ability from adjacent orchards and high reproductive rates, the management of the pest is complicated. Researchers have reported a host of control measures, including mechanical and cultural methods (manual removal of growing flushes, avoid pruning during active growth period to limit the flushing pattern, destruction of water shoots which attracts the pest for egg-laying, promoting flush growth in spring by fertilizer application in winter), host plant resistance (resistant germplasm like Trifoliate orange), biological (use of parasitoids like *Ageniaspis citricola* Loginovskaya, *Citrostichus phyllocnistoides* (Narayanan) and *Cirrospilus quadristriatus* (Subbarao and Ramamani)), semiochemicals and chemical control for the management of CLM[11].

Currently, there are no early pest detection methods for leaf miner and sampling of this insect to track infestation in the citrus groves of India. Often, infestation might have established in the foliage as soon as the first signs of its presence are visible. Use of pheromone lures for early detection of this pest has been reported from Florida, USA[12,13]. Three active compounds were also detected from female pheromone gland extracts of a Brazilian population of *P. citrella*: (*Z*, *Z*, *E*)-7,11,13-hexadecatrienal

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[$Z_7Z_{11}E_{13}$-16Ald], (*Z*, *Z*)-7,11-hexadecadienal [Z_7Z_{11}-16Ald] and (*Z*)-7-hexadecenal [*Z*7-16Ald] in the ratio 30 : 10 : 1 (ref. 14). The two-component lures are extremely attractive, attracting hundreds of moths per trap per night. Similar 3 : 1 binary mixture of $Z_7Z_{11}E_{13}$-16Ald and Z_7Z_{11}-16Ald was also found attractive to leaf miner field populations in Florida, USA and Vietnam respectively[15,16].

Till date, there have been no attempts to synthesize the sex pheromone of citrus leaf miner in India. Hence, an effort was made to synthesize the already identified pheromone components in collaboration with CSIR-Indian Institute of Chemical Technology (IICT), Hyderabad. Efficacy of indigenously synthesized pheromone-baited traps, viz. 3 : 1 blend of (*Z*, *Z*, *E*)-7,11,13-hexadecatrienal ($Z_7Z_{11}E_{13}$-16 Ald) and (*Z*, *Z*)-7,11-hexadecadienal (Z_7Z_{11}-16 Ald) was tested in <5-year-old orchards of acid lime cultivar. Our specific project objectives were: (1) to synthesize the major components in desired proportion of the sex pheromone of citrus leaf miner *P. citrella*; (2) to formulate the suitable dose and height at which the pheromone baited traps are to be placed in the tree canopy and (3) to compute the pheromone baited trap density for monitoring of CLM population during flushing seasons.

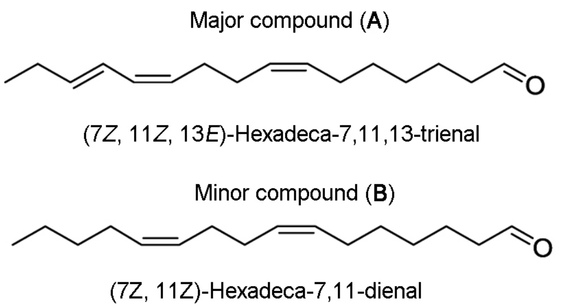
Materials and methods

The experiments were conducted at ICAR–Central Citrus Research Institute (CCRI) farm (21°11′5.86″ and 77°25′16.2″), Nagpur in *Citrus aurantifolia* (Christm) Swingle (acid lime) with 6 × 6 m^2 spacing from February 2014 to March 2016.

Synthesis of CLM female sex pheromone components

The female sex pheromone blend of CLM comprised of the following two pheromone components: **A** as major and **B** as minor in the ratio 3 : 1 respectively.

The synthesis of both components was from a commercially available chemical 3-octyn-1-ol (compound I; Scheme 1). The key intermediate (*Z*)-11-[(tetrahydro-2H-pyran-2-yl) oxy) undec-4-enal] (II) was prepared from compound I according to Scheme 1. From compound II, both **A** and **B** sex pheromone components were synthesized following Schemes 2 and 3 respectively[17].



Scheme 1.

Reagents and conditions: (a) $NaNH_2$, 1,3-diaminopropane, 80°C, 8 h. (b) *p*-TSA (para toluene sulphonic acid), DHP (dihydropyran), DCM (dichloromethane), 0°C, 0.5 h. (c) Li/NH_3, $Br(CH_2)_3OTBS$((3-bromopropoxy)(tert-butyl) dimethylsilane), 6 h. (d) TBAF (tetrabutyl ammonium fluoride), THF (tetrahydrofuran), 0°C. (e) Pd/$CaCO_3$, quinoline, EtOAc, H_2, 8 h. (f) IBX (2-iodoxy benzoic acid), CAN (acetonitrile), reflux, 1 h.

Reagents and conditions: (a) *n*-BuLi (*n*-butyllithium), (*E*)-bromo (pent-2-en-1-yl) triphenylphosphorane, THF, –78°C, 5 h. (b) *p*-TSA, MeOH, 0°C, 0.5 h. (c) IBX, ACN, reflux, 1 h.

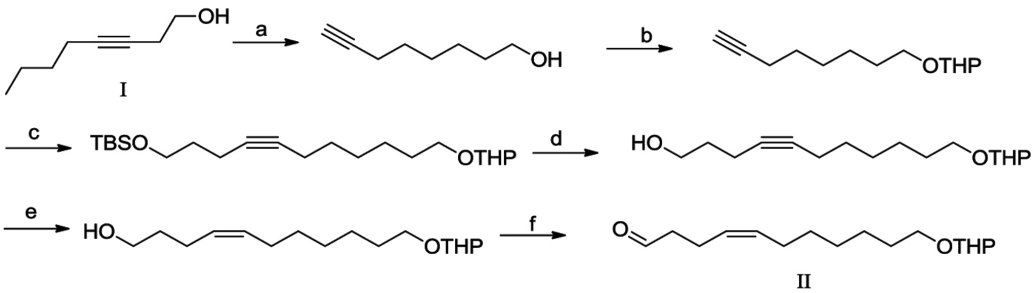
IR, ^{1}H, ^{13}C NMR and mass data of compound (A)

Molecular formula: $C_{16}H_{26}O$
IR (KBr): 3431, 3007, 2930, 2855, 2715, 1726, 1457, 1078, 983 cm^{-1}.
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.01 (t, 3H, $J = 7.4$ Hz), 1.34–1.42 (m, 4H), 1.63 (quint, 2H, $J = 7.4$ Hz), 2.00–2.06 (m, 2H), 2.06–2.17 (m, 4H), 2.18–2.26 (m, 2H), 2.42 (td, 2H, $J = 1.8$ and 7.4 Hz), 5.30 (dt, 1H, $J = 10.8$ and 7.4 Hz), 5.34–5.42 (m, 2H), 5.71 (dt, 1H, $J = 14.42$ for trans double bond proton), 5.96 (t, 1H, $J = 10.9$ Hz), 6.29 (ddq, 1H, $J = 15.2$, 12.10 for Cis double-bond proton), 9.76 (t, 1H, $J = 1.8$ Hz).
^{13}C NMR ($CDCl_3$; 125 MHz): $\delta = 202.4$, 136.2, 129.9, 129.1, 128.9, 128.8, 124.5, 43.7, 29.3, 28.6, 27.7, 27.2, 26.9, 25.7, 21.2, 13.5.
Mass: $[M]^+$ 234 *m/z* (Figure 1).

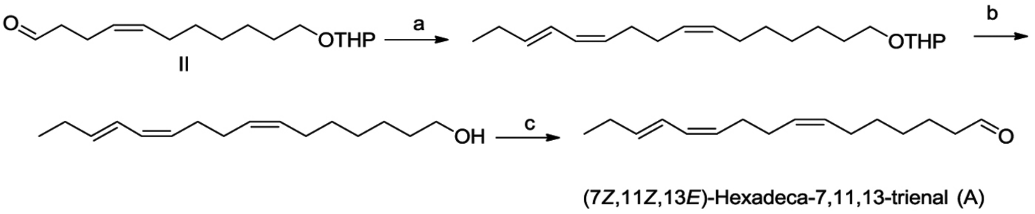
Reagents and conditions: (a) *n*-BuLi, bromo(pentyl)-triphenylphosphorane, THF, –78°C, 5 h. (b) *p*-TSA, MeOH, 0°C, 0.5 h. (c) IBX, ACN, reflux, 1 h.

IR, ^{1}H, ^{13}C NMR and MASS data of compound (B)

Molecular formula: $C_{16}H_{28}O$
IR (KBr): 2928, 1711, 1459, 1220, 968, 771 cm^{-1}.
^{1}H NMR (400 MHz, $CDCl_3$) δ 0.95–0.80 (m, 3H), 1.44–1.19 (m, 8H), 1.71–1.58 (m, 2H), 2.14–1.97 (m, 6H), 2.42 (td, $J = 7.4$, 1.8 Hz, 4H), 5.56–5.21 (m, 4H $J = 10.76$ Hz for cis double bond proton), 9.76 (t, $J = 1.8$ Hz, 1H).
^{13}CNMR ($CDCl_3$; 100 MHz): $\delta = 202.6$, 130.3, 129.7, 129.5, 128.9, 43.8, 31.9, 29.4, 28.7, 27.4, 27.3, 26.9, 26.8, 22.3, 21.9, 13.9.
Mass: $[M]^+$ 236 *m/z* (Figure 2).



Scheme 2.



Scheme 3.

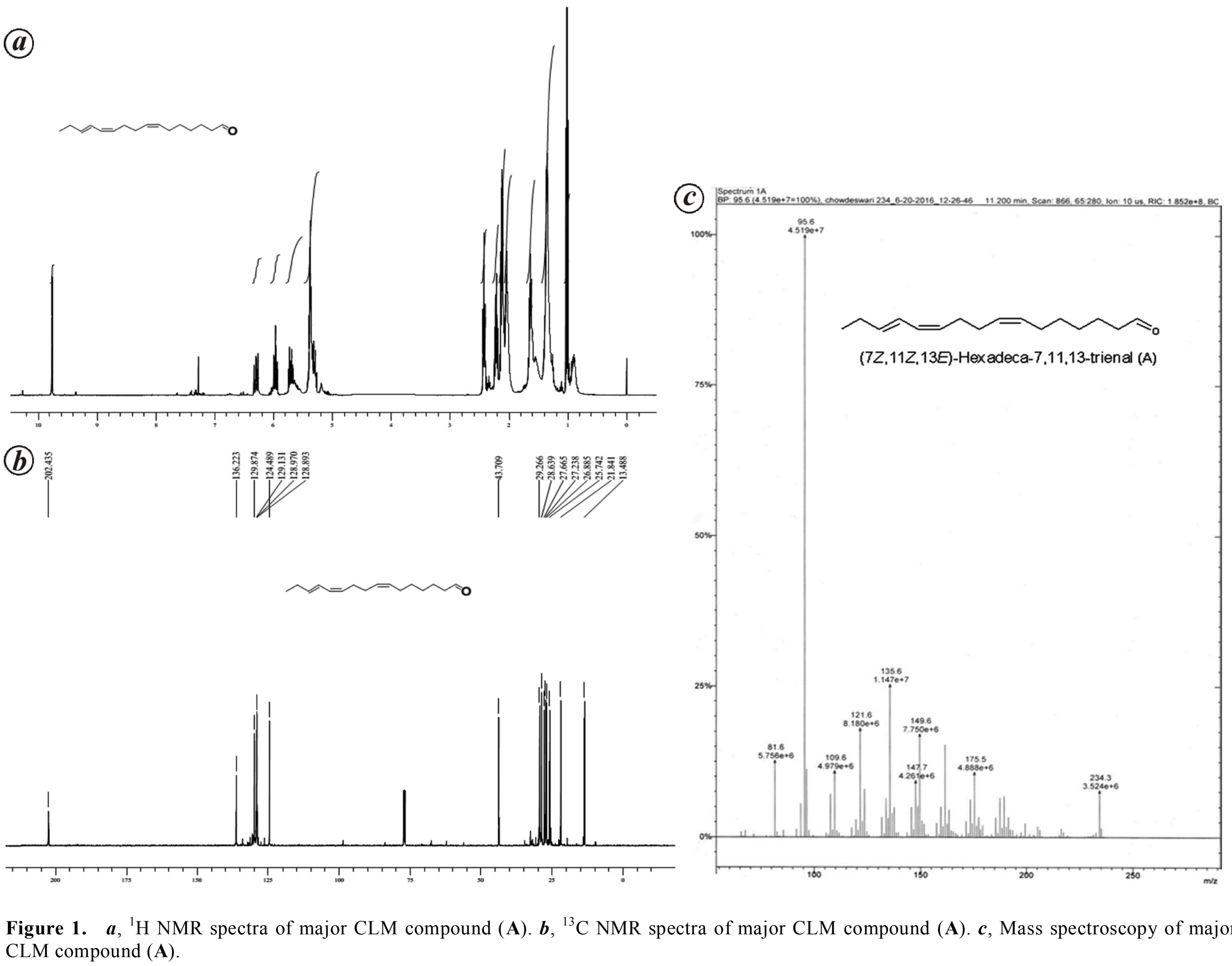


Figure 1. ***a***, [1]H NMR spectra of major CLM compound (**A**). ***b***, [13]C NMR spectra of major CLM compound (**A**). ***c***, Mass spectroscopy of major CLM compound (**A**).

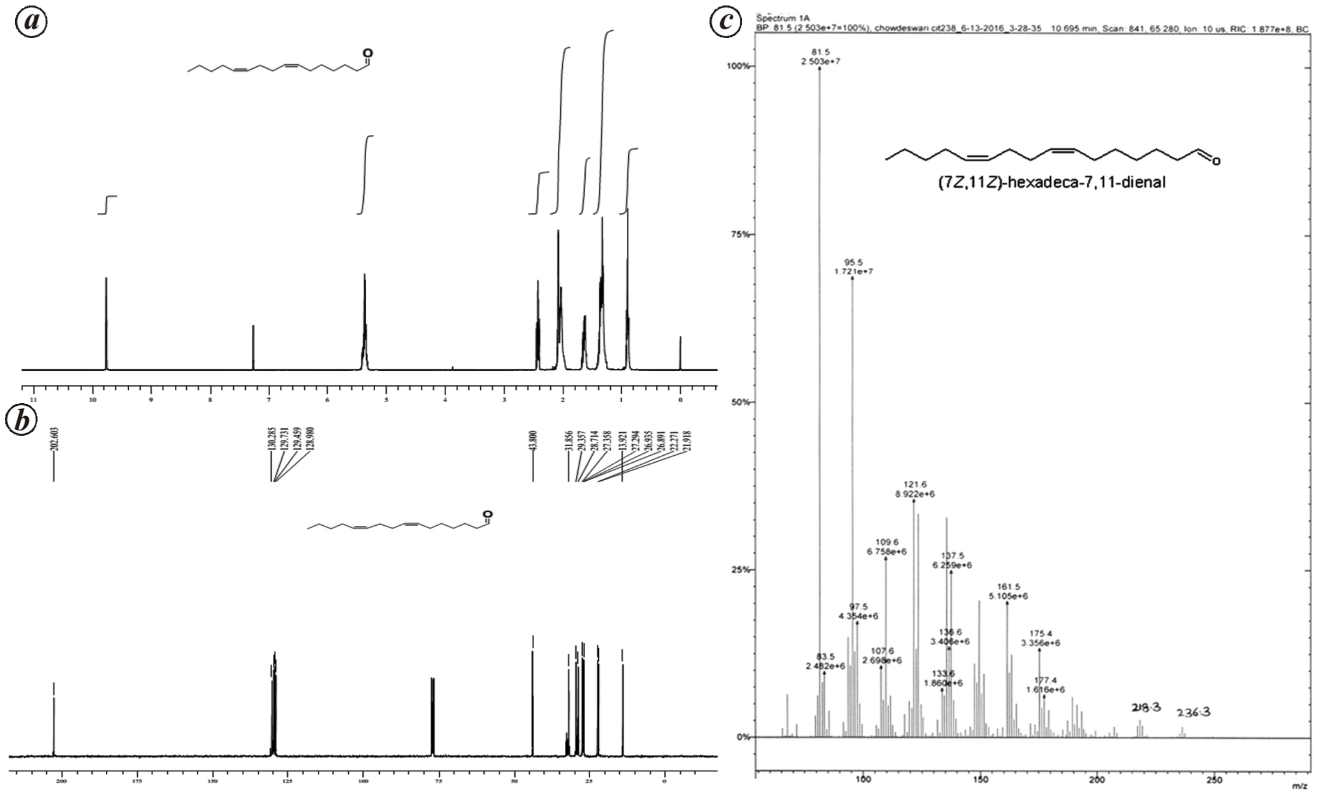


Figure 2. ***a***, [1]H NMR spectra of minor CLM compound (**B**). ***b***, [13]C NMR spectra of minor CLM compound (**B**). ***c***, Mass spectroscopy of minor CLM compound (**B**)

Lure dose

The study was conducted by evaluating the first indigenously synthesized pheromone lure for monitoring of *P. citrella*. For determination of optimal pheromone dose for male trapping, two doses, viz. 5 and 8 mg of *P. citrella* synthetic pheromone in a 3 : 1 blend of (*Z*, *Z*, *E*)-7,11,13-hexadecatrienal and (*Z*, *Z*)-7,11-hexadecadienal (synthesized at the Pheromone Application Technology, CSIR – IICT) were tested. Freshly synthesized pheromone components in hexane solution were impregnated into rubber septum dispenser and maintained at –20°C until use. Before installation in the field, lures were brought to room temperature 12–18 h before installation in the field. Pheromone dispensers were attached inside delta traps (26.5 cm × 18.5 cm × 11 cm) with graded yellow sticky card (20 × 15 cm^2) inside the traps. The pheromone baits were tested in <5-year-old acid lime orchards in the field. The trap catch per day was recorded.

Trap height and density

The second attempt was to arrive at the desirable height at which the pheromone-baited traps should be placed in the canopy. In the field, the traps hanged at mid-canopy (≃ 1.55 m from ground level in acid lime) and at canopy (≃3.14 m) height. Observations on the number of trap catches were made at weekly intervals.

After arriving at the desirable height, to assess the number of traps in unit area three treatments, viz. @ 10, 15 and 20 mg per 0.3 ha (block of 64 plants) were hung at mid-canopy level in the <5-year-old acid lime orchards, apart from control in completely randomized block design with seven replicates. Lures were changed after a period of 30–35 days when the trap catch was found to be unchanged for 5–7 consecutive days. The number of male moths captured per trap at weekly intervals was recorded.

Statistical analysis

Analysis by *t* test was used for comparison of factors with two levels for comparing the lure dose and trap height. When necessary, data were square root $(x + 0.5)$-transformed to stabilize variance and analysed by ANOVA. Unless otherwise noted, results are presented as mean ± SE; probability has been considered significant at a critical level of $\alpha = 0.05$.

Results

Identification of major components

For the structural conformation of compound **A** (7*Z*, 11*Z*, 13*E*)-hexadeca-7,11,13-trienal), we have used [1]H-NMR proton nuclear magnetic resonance system 400 MHz with internal standard as TMS (tetra methyl silane) and $CDCl_3$ (deuterated chloroform) as the solvent system and molecular mass spectrum studies using Varian gas chromatographic instrument (GC-MS-MS, 240-mass spectrum). The peaks shown in Figure 1 *c* depict the mass spectrum for compound **A** at the right side end peak (called the molecular ion peak in mass spectrometry) with mass value of 234 *m*/*z* and after loss of 139 *m*/*z* molecular weight, a peak appeared at molecular mass value at 95.6 *m*/*z* showing 100% relative abundance (called base peak). It was observed as a loss of fragment C_7H_{11} (CH_3–CH_2–CH=CH–CH=CH–CH_2) molecular ion and all remaining fragments showed a similar pattern loss of 14 *m*/*z*, which was assumed as methyline (–CH_2) group and loss of 12 *m*/*z* assumed as loss of carbon (–C) atom. Similarly, in Figure 2 *c*, the mass spectrum of compound **B** (7*Z*, 11*Z*)-Hexadeca-7,11-dienal) shows the right side end peak (molecular in peak) with exact mass value 236.3 *m*/*z*. The 100% relative abundance peak (base peak) was observed at mass value of 81.5 *m*/*z* which shows the loss of fragment 1-hexyne (CH_3–CH_2–CH_2–CH_2–C≡CH) molecular ion and all remaining peaks in the spectrum again showed a similar pattern loss of molecular ion 14, which was assumed as methyline (–CH_2) group and loss of 12 *m*/*z* assumed as loss of carbon (–C) atom.

Pheromone dose

The pheromone doses showed significant effect in male capture. Although male moth trap catch was obtained in both the doses, viz. 5 and 8 mg per lure, the greatest male captures were observed in traps baited with 5 mg (290.2 ± 10.01 adults/trap/day) than at 8 mg (183.5 ± 8.70 adults/trap/day; *t*-test significant at 1% and 5%; Figure 3).

Trap height and density

In the acid lime orchards, maximum trap catch was observed at mid-canopy height (*t*-test significant at 1% and 5%) than at canopy height (Figure 3). Male moth trap catch was significantly higher in acid lime blocks with 539.6 ± 8.44 and 667.15 ± 9.84 male catch/week/trap (($P \leq 0.014$); Pearson correlation = 0.0895) at canopy (3.14 m from ground level) and mid-canopy (1.55 m from ground level) height respectively.

Traps baited with 5 mg lure were further installed in the pre-bearing orchards of acid lime during two flushing seasons (August–December and January–March) during 2014–15 and 2015–16.

Monitoring of leaf miner population in pre-bearing orchards of acid lime

In the orchards, three to four times more trap catch was obtained in the installed traps irrespective of lure dose. During August–October 2014, maximum trap catch of 2053.85 ± 30.54, 1066.85 ± 33.08 and 340.00 ± 22.44 adults was observed from 4, 3 and 2 traps/block respectively. A second peak of maximum trap catch was observed during February–March 2015 (Figure 4). During 2016, mean monthly trap catch was 1400.17 ± 36.67, 873.64 ± 17.91 and 446.91 ± 8.78 adults from 4, 3 and 2 traps/block respectively. A maximum trap catch of 2343.14 ± 78.81 adults was collected in 4 traps/block in February 2016, followed by 1353.28 ± 29.79 and 1074.68 ± 22.28 adults in 2 and 3 traps/block respectively (Figure 5). A percentage reduction of 28.00 to 32.00 over control was recorded in the pre-bearing orchards with

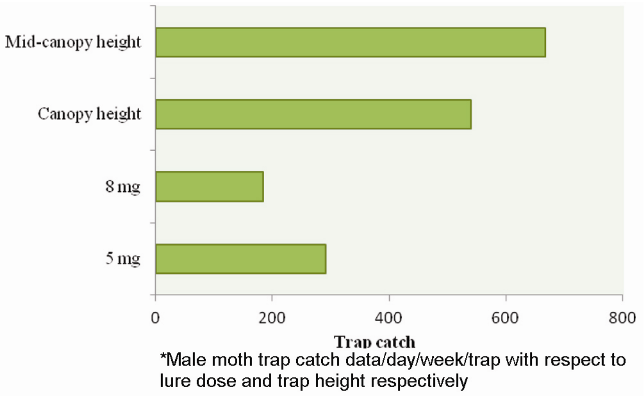


Figure 3. Trap catch of male moths at two different doses and heights in pre-bearing orchards of acid lime.

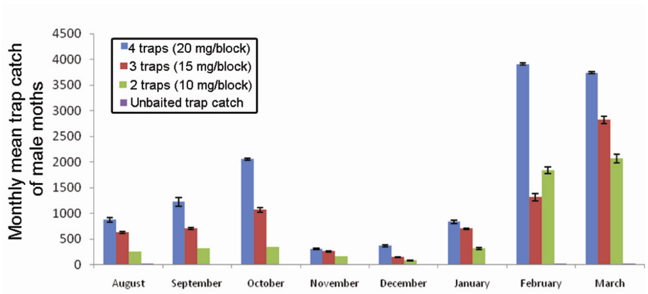


Figure 4. Trap catch of leaf miner male moths in different lure treatments in pre-bearing orchards of acid lime during 2014–15.

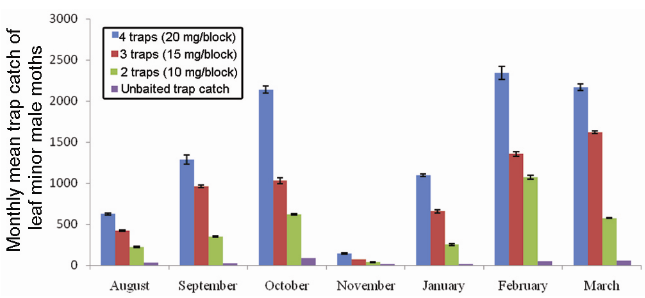


Figure 5. Trap catch of leaf miner male moths for different lure treatments in pre-bearing orchards of acid lime during 2015–16.

4 traps (20 mg) installed over a period of 6–7 months at the ICAR-CCRI farm.

Discussion

The above result demonstrates that the previously identified sex pheromone of the citrus leaf miner ((Z, Z ,E)-7,11,13-hexadecatrienal, (Z, Z)-7,11-hexadecadienal, 3 : 1) is an effective tool for monitoring *P. citrella* population[14,17]. We found that both the test doses, 5 and 8 mg attracted male moths, but trap catch was significantly more in 5 mg lure in the open field conditions. Lapointe *et al.*[18] confirmed that a 3 : 1 mixture of $Z_7Z_{11}E_{13}$-16Ald and Z_7Z_{11}-16Ald (67 µg dosage) loaded onto rubber septum lures attracts male *P. citrella* to traps (Pherocon 1C Wing Traps; Trece, Inc, Adair, USA) in Florida. Two doses, i.e. 10 and 100 µg of the synthetic sex pheromone – a 3 : 1 blend of (*Z*, *Z*, *E*)-7,11,13-hexadecatrienal and (*Z*, *Z*)-7,11-hexadecadienal attracted the greatest number of *P. citrella* males for over eight weeks in citrus groves[19]. However, mean total number of males caught in the higher dose (100 µg) was greater (46.85 ± 2.75) than in the lower dose (25.90 ± 6.86) over a period of 57 days. There was no significant difference in catches by traps loaded with low (50 µg) and high (500 µg) doses of pheromone[14]. It was observed during field evaluation of the sex pheromone of citrus fruit borer, *Ecdytolopha aurantiana* (Lepidoptera, Tortricidae: Olethreutinae) that traps baited with 1 mg of the pheromone blends captured more than those with 0.1 mg, both in 10 : 1 and 100 : 1 proportion[20]. Higher dose and slow releasing pheromone dispenser seem to be effective in attracting the moths over a period of time.

In the present study, male moth catch was higher when the pheromone baited traps were placed at mid-canopy height within the tree canopy[12]. Using the same pheromone components in delta traps of the present study in mature citrus trees, Stelinski and Rogers[12] observed that male capture was highest at 2 m above ground level within the tree canopy and at 0.6 m above ground level on the perimeter of the tree canopy. Pheromone traps placed within and near the tip of the tree canopy in apples captured more male *C. pomonella* (apple codling moth) than those placed at the bottom edge of the tree canopy[21,22]. Contrary to our observations, Lapointe *et al.*[18] observed that trap height at 0.7, 1.3 and 2 m did not significantly affect the daily rate of male catch in traps. The discrepancy between these two studies is likely due to the smaller tree height (2.0 m) and lesser range of heights investigated (1.3, 1.7 and 2.0 m) by them[18] than in the present study. From a practical monitoring perspective also, placing pheromone-baited traps at mid-canopy levels of mature citrus trees could result in optimal catch of male *P. citrella*.

CLM infestation in central Indian conditions increased during the first week of August after the rains due to emergence of young flush leaves till early December, whereas the minimum per cent infestation was observed during January and May–June[23]. During our monitoring with the indigenous synthetic pheromone lure, maximum trap number and per cent reduction in leaf miner infestation were obtained in orchards where pheromone-baited traps @ 20 mg were placed in comparison to 10 and 15 mg. Trap catch data were also in concurrence with the population fluctuation (per cent infestation) of leaf miner (Figures 6 and 7), substantiating the fact that the indigenous pheromone works well under Indian conditions. A trap catch of over 1700 of *P. citrella* moths per sticky card deployed for 9 days (192 moths/day), which were loaded with two constituents incorporated in grey halobutyl rubber septa in a 3 : 1 ratio has been reported[15].

The monitoring of citrus leaf miner field population with synthetic sex pheromone traps can rationalize its control, reducing insecticide application costs and other hazardous environmental impacts. Much work has not been done in India with reference to the synthesis and use of the pheromone component for management of CLM. Although several outstanding successes have proved the potential of pheromone technology, use of semiochemicals is still in its early stage in India. Given the importance of

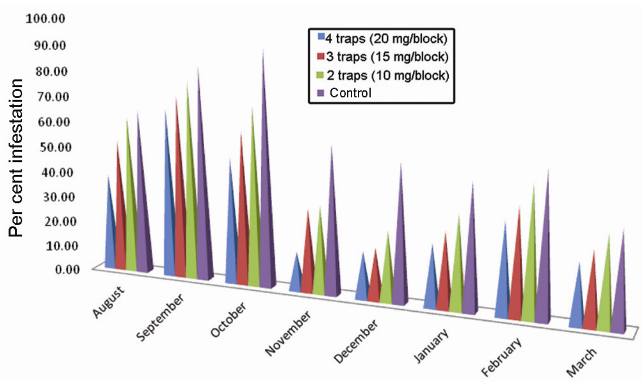


Figure 6. Per cent infestation of leaf miner for different lure doses in pre-bearing orchards of acid lime during 2014–15.

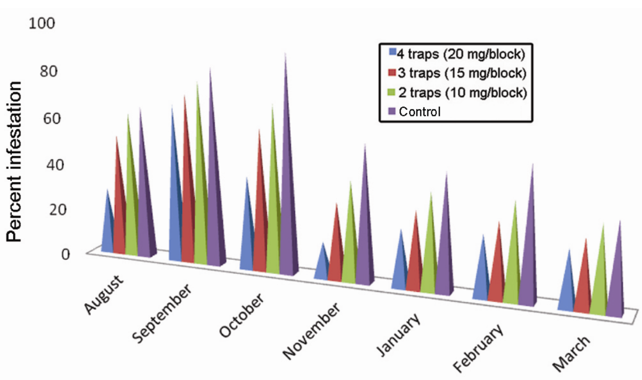


Figure 7. Per cent infestation of leaf miner for different lure doses in pre-bearing orchards of acid lime during 2015–16.

the *P. citrella* pest, the indigenously synthesized sex pheromone might be useful in citrus seedling nurseries as well as establishment of young orchards during the initial period of growth. A prophylactic spray with insecticides could be decided by observing the leaf miner population in the traps installed well in advance of the population reaching an alarming rate on the young flush leaves. Thus, control of *P. citrella* with pheromone traps reduces the number of insecticide sprays for leaf miner management in citrus groves. Furthermore, it will provide a valuable tool for following the population cycle and estimating the population size of citrus leaf miner infestation. We anticipate that after one more year of evaluation of the sex pheromone, the lures could be commercialized for use by the citrus growers in central India.

1. Hoy, M. A. and Nguyen, R., Classical biological control of the citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae): theory, practice, art and science. *Trop. Lepid Res*., 1997, **8**(1), 1–19.
2. Clausen, C. P., Two citrus leaf miners of the Far East. USDA, Washington, DC, Tech Bull, 1931, vol. 252, pp. 1–13.
3. Clausen, C. P., The citrus insects of tropical Asia. US Department of Agriculture, Washington, DC. *Cir*, 1933, **266**, 1–35.
4. Stainton, H. T., Descriptions of three species of Indian micro-Lepidoptera. *Trans. Entomol. Soc. London*, 1856, **3**, 301–304.
5. Sharma, D. R., Singh, S. and Arora, P. K., Population dynamics and management of citrus leaf miner, *Phyllocnistis citrella* Stainton on Kinnow mandarin. In National Seminar on Integrated Production and Post Harvest Management of Tropical Fruit, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia district, West Bengal, 11–12 April 2006, p. 44.
6. Shivankar, V. J., Rao, C. N. and Singh, S., Citrus insect pest management. Manual No. 1, National Research Centre for Citrus, Nagpur, 2002. p. 11.
7. Sohi, G. S. and Sandhu, M. S., Relationship between citrus leaf miner (*Phyllocnistis citrella* Stainton) injury and citrus canker (*Xanthomonas citri* (Hasse) Dowson) incidence on citrus leaves. *J. Res. Punjab Agric. Univ*., 1968, **5**, 66–69.
8. Gottwald, T. R., Graham, J. H. and Schubert, T. S., An epidemiological analysis of the spread of citrus canker in urban Miami, Florida and synergistic interaction with the Asian citrus leaf miner. *Fruits*, 1997, **52**, 371–378.
9. Achor, D. S., Browning, H. W. and Albrigo, L. G., Anatomical and histological modification in citrus leaves caused by larval feeding of citrus leaf miner (*Phyllocnistis citrella* Stainton). In Proceedings of the International Conference Citrus Leaf Miner (ed. Hoy, M. A.), University of Florida, Florida, Gainesville, USA, 1996, p. 69.
10. Shivankar, V. J., Ghosh, D. K., Das, A. K. and Rao, C. N., *Tropical and Subtropical Citrus Health Management*, Satish Serial Publishing House, New Delhi, 2014.
11. Shivankar, V. J., Rao, C. N. and Singh, S., Citrus leaf miner (*Phyllocnistis citrella* stainton) management: a review. *Agric. Rev*., 2000, **21**(3), 205–210.
12. Stelinski, L. L. and Rogers, M. E., Factors affecting captures of male citrus leaf miner, *Phyllocnistis citrella* Stainton, in pheromone-baited traps. *J. Appl. Entomol*., 2008, **132**, 143–150.
13. Kawahara, A. Y., Jones, M., Jia, Q., Lapointe, S. L. and Stansly, P. A., A synthetic pheromone for *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) attracts multiple leaf miner species. *Fla. Entomol*., 2003, **96**(3), 1213–1216.
14. Leal, W. S., Parra-Pedrazzoli, A. L., Cossé, A. A., Murata, Y., Bento, J. M. S. and Vilela, E. F., Identification, synthesis and field evaluation of the complete sex pheromone from the citrus leaf miner, *Phyllocnistis citrella*. *J. Chem. Ecol*., 2006, **32**, 155–168.
15. Lapointe, S. L. and Leal, W. S., Describing seasonal phenology of the leaf miner *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) with pheromone lures: controlling for lure degradation. *Fla. Entomol*., 2007, **90**(4), 710–714.
16. Van Vang, L. *et al*., 7,11,13-Hexadecatrienal identified from female moths of the citrus leaf miner as a new sex pheromone component: synthesis and field evaluation in Vietnam and Japan. *J. Pest. Sci*., 2008, **33**, 152–158.
17. Moreira, J. A., Mcelfresh, J. S. and Millar, J. G., Identification, synthesis, and field testing of the sex pheromone of the citrus leaf miner, *Phyllocnistis citrella*. *J. Chem. Ecol*., 2006, **32**, 169–194.
18. Lapointe, S. L., Hall, D. G., Murata, Y., Parra-Pedrazzoli, A. L., Bento, J. M. S., Vilela, E. F. and Leal, W. S., Field evaluation of a synthetic female sex pheromone for the leaf mining moth *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Florida citrus. *Fla. Entomol*., 2006, **89**, 274–276.
19. Parra-Pedrazzoli, A. L., Leal, W. S., Vilela, E. F., Mendonça, M. C. and Bento, J. M. S., Synthetic sex pheromone of citrus leaf miner in Brazilian citrus groves. *Pesqui. Agropecu. Bras.*, 2009, **44**(7), 676–680.
20. Leal, W. S., Bento, J. M., Murata, Y., Ono, M., Parra, J. R. P. and Villela, E. F., Identification, synthesis, and field evaluation of the sex pheromone of the citrus fruit borer *Ecdytolopha aurantiana*. *J. Chem. Ecol.*, 2001, **27**(10), 2041–2051.
21. Thwaite, W. G. and Madsen, H. F., The influence of trap density, trap height, outside traps and trap design on *Cydia pomonella* (L.) captures with sex pheromone traps in New South Wales apple orchards. *J. Aust. Entomol. Soc.*, 1983, **22**, 97–99.
22. Howell, J. F., Schmidt, R. S., Horton, D. R., Khattak, S. U. K. and White. L. D., Codling moth: male activity to pheromone lures and pheromone-baited traps at different elevations within and between trees. *Environ. Entomol*., 1990, **19**, 573–577.
23. Alexander, A., Kuttalam, S. and Rao. C. N., Effect of weather parameters on incidence of citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its natural enemies in three commercially grown citrus cultivars. *Entomon*, 2014, **39**(2), 67–76.

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